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PRINCIPAL INVESTIGATOR: Margo N. Woods, D.Sc.

CONTRACTING ORGANIZATION: Tufts University School of Medicine
Boston, Massachusetts 02111

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Women at high risk for breast cancer who are experiencing menopausal symptoms of hot flashes are being recruited for our study in Boston and New York. Breast Health Clinics and Women's Clinics are being used to inform women about the study. In Boston, we have recently used radio advertising to locate our study population which has been highly successful. Women with ≥ 5 symptoms per day are randomized, using a double-blind, cross-over study design, to receive either a placebo bar or soy supplement bar for 3 months with one month of wash-out between protocols. Two blood samples are taken at baseline and during the last week of each of the two 3-month study periods. Serum levels of estrone, estradiol, estrone-sulfate, androstenedione, and follicle stimulating hormone are measured. Subjects keep a daily symptoms diary for the entire seven month study. A matched group of control women with ≤ 2 hot flashes/day are also being studied. This study proposes to determine: 1.) if a dietary soy supplement (containing 45 mg of phytoestrogens) relieves menopausal hot flash symptoms, 2.) if a dietary soy supplement bar affects hormone levels, and 3.) the relationship between menopausal symptoms and hormone levels.

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Mary N. Woods October 25, 1996

PI - Signature Date

Annual Report

September 30, 1995 - September 29, 1996

Effect of a Soy Dietary Supplement on Menopausal Symptoms and Hormones in Women at High Risk of Breast Cancer

Principal Investigator: Margo N. Woods, D.Sc.

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1. INTRODUCTION

Phytoestrogens which are found in a number of fruits, vegetables, and grains, but occur in the highest concentrations in soy (1,2,3), have demonstrated both estrogenic and antiestrogenic activity (4). Although they are not steroids, structurally they resemble estrogens and they bind to estrogen receptors (5). Their estrogenic activity has been estimated at .002 and .001 (1/500 and 1/1,000) that of estradiol (6,7). Animal studies have reported a protective effect of phytoestrogens in mammary tumor models (8,9,10) while epidemiological studies in the Asian population have demonstrated an inverse relationship between soy intake and breast cancer (11,12,13). This has expanded the debate on identification of the environmental factors contributing to the observed lower risk of breast cancer in the Asian population to include intake of fat, fiber, and phytoestrogens (14,15).

These interesting effects of soy have prompted dietary soy studies in premenopausal women (16,17,18) measuring serum estrogens and gonadotropins and cycle length. A cross-cultural study on menopause (19) reported lower levels of hot flashes in Japanese women compared to women in North America which further implicated the possibility of soy intake in the Asian diet having biological estrogenic effects. It is estimated that in this population the daily intake of phytoestrogens is approximately 40 mg/day (20). Soy supplementation studies in post-menopausal women (21,22,23) have therefore investigated serum hormone and gonadotropin levels, vaginal cytology, and menopausal symptoms.

Cassidy, et al. (16,17) studied premenopausal women who were given foods containing 45 mg of phytoestrogens/day for one month and reported an increase in estradiol in the follicular phase of the cycle, and a decrease in midcycle surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH). An increase in the length of the follicular phase of the menstrual cycle was also observed. Lu, et al. (18) also studied premenopausal women but gave foods containing 200 mg/d of phytoestrogens, for one month, and reported a decrease in estradiol. LH and FSH were not measured. The differences in estradiol responses observed between the two studies could be due to the difference in phytoestrogen intake since a biphase response to phytoestrogens has been reported (24,25). Wilcox, et al (21) and Múrkies, et al (23), studied post-menopausal women consuming 45 g of soy flour/day (estimated phytoestrogen intake of 80-138 mg/d) for 2 weeks or 12 weeks respectively. Baird, et al (22), also studying post-menopausal women used foods containing 165 mg/day of phytoestrogens (in texturized soy vegetable protein) for one month and observed no change in estradiol, LH, or FSH. Both Wilcox, et al (21) and Baird, et al (22) reported changes in vaginal cytology that indicated an estrogenic effect of the soy products. Múrkies, et al (23) reported a decrease in menopausal symptoms in women while on the soy flour. However, a matched group consuming wheat flour also reported a decrease in menopausal symptoms, and there was no significant difference in reported hot flashes between the two groups after 12 weeks.

In order to test the effect of phytoestrogen intake on menopausal symptoms of hot flashes, we designed a double-blind study using a dietary supplement bar in which menopausal women consume 45 mg of phytoestrogens/day or a placebo containing casein protein with 0 mg of phytoestrogen. Participants are randomly assigned to consume either bar for 3 months with one month of wash-out,

followed by another 3 months of the alternate bar. Menopausal symptoms are tracked and recorded daily, and hormone and gonadotropins are determined at baseline and at the end of each dietary phase.

Primary study hypothesis: Consumption of 45 mg/day of phytoestrogens in a soy product will decrease the number of menopausal hot flashes reported by menopausal women with > 5 hot flashes/day and will decrease gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) after three months.

Secondary study hypotheses: Women reporting higher numbers of hot flashes (> 5/day) will have lower estrogen and higher FSH levels than women reporting lower numbers of hot flashes (< 2/day). Frequency of hot flashes will correlate to serum levels of estrogen and gonadotropins.

2. BODY

A. Experimental Methods

Study design: *Intervention Group:* Women reporting hot flashes are recruited into the study and during a one week baseline period record the number, time, and severity of each hot flash. Baseline blood is obtained to determine estrogen and gonadotropin levels. Those women who report, on average, 5 or more hot flashes per day are randomized into the double blind dietary intervention study in which they were asked to consume two dietary bars per day for a period of 3 months. One arm of the group consumes two dietary soy bars/day that contain 45 mg of phytoestrogens/day and the other arm consumed two dietary casein bars/day with < 1 mg of phytoestrogen/day. A one month washout period is followed by 3 months of the alternate dietary bar. During the last week of each 12 week study period two blood samples are obtained for determination of serum estrogens and gonadotropins. Women record daily hot flashes during the entire 7 months of the study.

Control Group: Women experiencing <2 hot flashes/day participate in a one week study to obtain information about hormones, dietary intake, and menopausal symptoms.

Subjects: Women, 45-59 years of age, at increased risk for breast cancer, who have not had a period for 6 or more months are recruited. Women currently taking hormones, steroids or other medications that interfere with hormone metabolism are excluded. Women who had previously taken hormones must be off them for at least six months prior to beginning the study. Women are excluded if they have undergone surgical menopause with no intact ovaries or currently have a high intake of soy products (greater than 2 times/week).

Dietary supplements: The soy supplement consists of a dietary bar that contains 196 kcal, 15.1 g protein, 1.3 g fat, and 24 mg of phytoestrogens (genistein and diadzien). The phytoestrogens are present in the soy protein fraction. The placebo bar uses casein protein in place of the soy protein and contains 194 kcal), 16.4 g protein, 0.9 g fat, and < 1 mg of phytoestrogens. Subjects report whether they had consumed their two dietary bars each day as part of their daily recording of menopausal symptoms.

Menopausal symptoms: Subjects are given a menopausal symptom diary to keep daily records of the number of symptoms they have during the day and at night (night sweats). During the final week of each phase of the study, they are also asked to record the time of each hot flash and its severity (mild, moderate, or severe).

Hormone determinations: Blood samples are collected on two days, within one week of each other, after consuming the supplement bars for 11-12 weeks. Samples are centrifuged at 1400 x g rpm for 22 minutes at 4 C and the serum is stored at -70 C. Individual samples from each day are run in duplicate. An average of the two determinations is used in the analysis. Serum hormone measurements are carried out for estrone, estradiol, estrone-sulfate, androstenedione, testosterone, sex hormone binding globulin (SHBG), and free estradiol using radioimmunoassays involving solvent extraction and celite chromatography, as previously described (26,27). All samples are coded using a random number system and blinded duplicates were inserted as a quality control measure. LH and FSH are determinated using a double-antibody radioimmunoassay utilizing a kit obtained from Radioassay Systems Laboratory, Inc., Carson, CA.

B. Results

1. Current Recruitment Status

a.) Boston site

Our recruitment goal for the entire 4 year project is to enroll N= 50 women with high risk for breast cancer who are currently reporting a high level of hot flashes ($\geq$5/day) and N=50 women at high risk who are reporting a low number of hot flashes ($\leq$2/day). In order to complete the study in a timely manner this recruitment goal should be met by September 1997. Over the three year period this translates to approximately 17 women per year from each group. The current status of the recruitment program is as follows:

I . Advertisement of the study

The researchers have a long history of success in recruiting women for intervention studies related to diet, hormones and risk of breast cancer. Recruitment plans included using the New England Medical Center's "Breast Health Clinic" as a site for recruitment. We obtained the mailing list of all patients who have attended the clinic and sent two mailings to all women in the appropriate age group regardless of their history of breast cancer. This was done within the first six months of the study. Two 30 minute presentations were also made to the staff at the clinic in the first year. At the same time we put up posters advertising our study throughout the New England Medical Center Complex which is extensive and advertised in the three newspapers or bulletins distributed in the Tufts-New England Medical Center complex. This was followed by letters to over 200 OB/GYN physicians in the Boston area explaining our study and providing brochures on the study for distribution in their offices. A recruitment advertisement was also put into the Boston Globe and 5 suburban newspapers.

In the beginning of the this year (the second year) we expanded our recruitment efforts to identification of menopausal clinic sites in the area. Fifty sites were located and 10 agreed to have posters and brochures displayed at their clinics. Special presentation were made at 8 such clinics in the Boston area. These presentations were usually to both the lay public and the medical staff who were seeing women in this age category. Special sites included:

1. Breast Health Clinic, New England Medical Center, Boston
2. Emerson Hospital, Concord
3. Metro West Medical Center, Framingham (Annual Cancer Conference)
4. Women's Health Pavilion, Milford Hospital
5. Lahey Clinic North, North Shore, Peabody (Women's Health Seminar)
6. Spence Center for Women, Cambridge
7. US Army Natick Research Laboratory at Natick, Seminar
8. Newton-Wellesley Hospital, OB/GYN Dept.

At this time we also increased our catchment area to include the area between Route 128 and Route 495. Additional outlying suburban newspapers were used to advertise our study. Advertisements have been run in over 30 newspapers. In July, 1996 we decided to pursue radio advertisement as a way of reaching a larger audience. Thirty six - one minute informational spots were aired in a five day period on a local talk show radio station. This lead to a record number of responses, more than 300 telephone calls within one week.

ii.) Recruitment status

Number of phone calls received from prospective study candidates.....	600
Number of study packets sent to eligible women as determined by telephone interview.....	50

High level of hot flashes

Number of women enrolled in the study.....	24
Number of women in Phase I	14
Number of women in Washout, Phase II.....	6
Number of women in Phase III.....	3
Number of women who have completed the study.....	4

Low level of hot flashes

Number of women enrolled in the study	2
Current number of phone enquires being pursued (as of September 23rd).....	100

We are currently scheduled for approximately 10 subject visits/wk.

iii.) Future plans

We intend to continue the use of radio advertisement to reach our target population. This will probably consist of a two day series of radio "spots" every two months. In addition we will pursue public service and medical news TV spots for the fall. In the past we have had great interest from the local television stations but each wanted to interview an actual study participant. Until this time we could not find a participant who was willing to be interviewed on TV. We now have a few women who would consent to do this. Other issues regarding recruitment are addressed under part C (Discussion of Problems and Issues).

b.) Memorial Sloan-Kettering site

Recruitment activities in the Special Surveillance Breast Program (SSBP) located in the Memorial Sloan Kettering Breast Center commenced in October, 1995. The population attending SSBP includes women of all ages at increased risk of breast cancer due to either having a family history of the disease or having a history of breast surgery resulting in the diagnosis with premalignant lesion. Patients are followed every three to six months. More than 400 women are registered with the SSBP program and approximately 50 new patients have registered in the past year.

Efforts to promote participation of patients in the study have been focused on physician and patient awareness within the MSK Breast Center. A brochure about the study is displayed in the waiting areas an on bulletin boards in the Center. Physicians have referred patients to the study coordinator. During the past 12 months, more than 250 patient records have been reviewed with the attending surgeon at the time of the patients' appointments. Of these four women have enrolled in the intervention arm; one patient has completed Phase I but was unable to complete Phase III; one woman is currently in Phase II; one has completed the first 2 months of Phase I and the fourth woman will begin Phase I on September 27th. All three subjects with low levels of symptomatology (controls) have completed the study protocol.

Recruitment of study subjects has been negatively affected by the increased use of hormone replacement therapy among these patients who are known to be at elevated breast cancer risk. This reflects a recent change in medical recommendations from gynecologists. Among the SSBP patients who meet the soy study requirements, 26 are enrolled in the Tamoxifen Prevention Trial making them ineligible for recruitment. Twenty-nine screened women were found to have prior hysterectomy and/or oophorectomy and sixty-seven within the age range for recruitment reported regular menstrual cycles. Seven women were not interested in participating because they considered the protocol was too time consuming.

Additional avenues are now being explored for recruitment. Surgeons affiliated with Mount Sinai Medical Center and Beth Israel Medical Center have been asked to participate in our recruitment efforts. Both institutions have recently opened breast centers offering screening to women at elevated risk of breast cancer. In addition, Memorial Sloan Kettering Cancer Center has recently purchased the Guttman Breast Cancer Screening Center, a facility located a distance from the

hospital that screens approximately 200 women daily. Meetings with the medical director indicate that patients will be provided information about the study and the study coordinator will visit the screening center on a regular basis to enhance recruitment efforts.

Additional recruitment to the soy study is anticipated through publicity of the project to members of high risk families enrolled in the Metropolitan New York Registry of Breast Cancer Families recently funded by the National Cancer Institute. This Registry based at MSKCC is a multi-institutional collaborative effort to create a resource for genetic epidemiologic studies. In addition to family history among participating family members, data collection includes reproductive information and hormone use. Participation in the soy study will be offered to all women of appropriate ages who are experiencing extensive menopausal symptoms and do not wish to take hormone replacement therapy.

2. Data Entry

The data entry programs for our study are complete, and we will begin data entry in November using Paradox software and data entry forms that duplicate the hard copies of the study forms. All data will be double entered and checked for congruency. We have available expertise in our unit for methodology in data entry and subject tracking since we are engaged in a large longitudinal program project on Nutrition and HIV. We have conferred with the data management director on our procedures and will use some of their data entry personnel for our study.

C. Discussion of Problems and Issues

1. Recruitment

a). Boston site

A major issue for the study has been the recruitment of subjects. Our study investigators, staff and recruiters have worked hard and with considerable ingenuity to locate sites to advertise and recruit prospective subjects. The population identified in the study, women at increased risk of breast cancer, was originally chosen because of the medical position at the time of the grant submission, that women at increased risk for breast cancer should not use HRT. Women with breast cancer were not identified because discussions with oncologists and OB/GYN indicated that physicians would be reluctant to have women on tamoxifen or other chemopreventive agents take a soy product because of the unknown nature of the effect of the drug combination. In contrast, a large number of peri-menopausal and post-menopausal women were being encouraged by their physicians to start HRT and we believed that it would be difficult to recruit in this population except for that small group of women who were interested in only a "more natural" treatment for menopause. National data on the use of HRT indicate that approximately 30% of women are taking HRT. In some metropolitan areas this percent increases and in California it is estimated that 80% of women entering menopause choose to select HRT. In the short time period that has elapsed from the time of the grant submission until the current date, a significant shift has taken place in the Boston area on physician recommendations on HRT. Currently, physicians are encouraging women at increased

risk or with breast cancer to select HRT. We believe this has had a significant impact on our recruitment using our standard approach of contact with breast health clinics and physician practices. In addition, the Women's Health Initiative in Boston, has culled this age group for recruitment to their study.

Our recruitment efforts were definitely unsatisfactory until we decided to advertise more directly with the public and increase the money spent on recruitment. Increasing the size and placement of our advertisements in the Boston and suburban populations resulted in a significant increase in telephone calls regarding our study. Use of radio spots ($2,000 for 36 one minute messages) resulted in an overwhelming public response and necessitated hiring a person for 8 days to return the phone calls to carry out preliminary screening. We believe we will easily meet our recruitment objectives and be able to go beyond our original goal of 50 women at increased risk for breast cancer.

b.) New York site

The Sloan-Kettering Clinic which only sees women at increased risk for breast cancer also experienced resistance from the physicians and patients for recruitment. This was related in part to the increased use of HRT in their patients and to the physician's negative response to the soy bar since it was high in calories. The addition of two new clinics for recruitment should improve recruitment in the next year but general advertising to the public will also have to be considered in light of the response in Boston.

***2. Changes to the protocol of the study as originally submitted*:**

a. Stipend. The stipend to the women went from $120 to $350 for the 7 month study. This increase was necessary because of other competing studies in the area that were paying the higher amount for 3-4 months of study. This will result in budget problems which we are addressing by hiring work-study students to decrease our expenses in personnel.

b. Urine Collection. We are currently collecting only the first morning urine void instead of the 24 hour urinary sample as proposed in the contract. This sample is for urinary phytoestrogens and a morning void will be sufficient to determine compliance to the protocol since increases in urinary phytoestrogens are so dramatic. If other laboratory determinations are to be carried out on the urine this will have to be reconsidered.

c. Washout Phase. The washout phase, Phase II, was initially proposed as a time when all subjects consumed the placebo bar. A break from the consumption of the dietary bar was considered to be important to the subjects and therefore we changed the protocol. This would also make it less likely for the subjects to know if they are on the placebo since they would know the washout was the placebo and Phase III bars could be compared to this Phase II placebo. The soy and placebo bars are very similar but some subtle differences are present which would become easier to identify with a clear placebo washout. This decision was made before any subjects were recruited.

d. Advertising Budget. The original advertising budget was inadequate for the necessary recruitment strategies that we needed to employ. The importance of trying the expensive radio and newspaper ads was discussed and supported. The study funds were supplemented with department funds for this purpose.

e. Eligibility Status. A re-evaluation of eligibility requirements took place in the first six months of the study prior to recruitment and slight modifications were made. The age range was measured from 48-58 years of age to 45-59 years of age. The definition of post-menopausal was changed from "no periods for at least one year" to "no periods for at least six months and an FSH/LH ratio > 1".

f. Concurrent Controls. We will seek agreement from the control women (those with ≤ 2 hot flashes/day) to track their symptoms for 3 months to obtain data on "usual" fluctuations in symptoms over this time period. This additional request would be submitted to our human research board for approval. Women would not be disqualified from the control group if they did not agree to this additional monitoring. The desirability of concurrent controls was discussed at the site visit, and we believe it might be possible to obtain their data without undue inconvenience to the control group.

g. Biostatistician. We have requested that the function of biostatistician be transferred from the Sloan-Kettering to Tufts University School of Medicine, Boston and be carried out by Donna Spiegelman (D.Sc.). This request with her C.V. has been forwarded previously, and Dr. Spiegelman participated in the site visit on October 9, 1996.

h. Nutrient Database. We are currently using the Minnesota Nutrient Database instead of the Tufts Nutrient Database due to guarantee of timely updates.

3. Clarification of protocol

Since some of these issues were unclear to the site visit personnel, we have included them in our yearly report and inserted them into our Manual of Operation.

a. Weighing of subjects. Subjects are being weighed when they are screened and at each time point that blood is collected.

b. Exercise Questionnaire. Subjects are asked to let us know if their exercise activities change. They are asked to fill out the activities sheet at baseline and when they come in for blood collection.

c. Definition of High Risk. The wording in the grant is not exact on this point, and we adopted the following criteria at the beginning of the study:
1. First degree relative with breast cancer or two second degree relatives with breast cancer
2. Two or more benign breast biopsies

3. One breast biopsy with diagnosis of atypical proliferative breast disease
4. Diagnosis of Lobular Carcinoma In Situ (LCIS)

d. Source of soy bars. Protein Technology, Inc. are providing us with the dietary soy bars for the duration of the study. The bars contain 190-200 kcalories/bar and 24 mg of phytoestrogens. Subjects are requested to have two/day and are counseled on how to incorporate them into their diet so as to maintain their current weight. The ingredients and nutrient specifications are included in the Appendix C.

e. Batching of blood samples. Specifications on how and when the blood samples would be sent for analysis were not specific in the contract. We will send the samples in batches so that all of a subject's blood samples will be in the same batch. This will decrease variation due to difference between batches. Therefore, samples are kept frozen at -70° C for 6-7 months prior to sending them for hormone analysis.

f. Data entry. We plan to start entering study data in the next month and will use double entry. Our data entry system has been developed on Paradox and will be transferred to our biostatistician for analysis on SAS.

g. Collection of two blood samples, within one week. In last year's report, the usefulness of taking two blood determinations within one week was questioned due to variability of the values over this time period (1-7 days). Since these women are not having periods, we expect no difference in hormone values one to seven days apart, however, during the peri-menopausal period, it is known that hormone values do vary. We will be able to compare these hormone values to determine the stability of the hormone levels in this population. The double sampling is more rigorous than one blood sample although it does increase costs for hormone determinations. See section 4a. on recommendation that we test serum phytoestrogen levels in a subset of this study population.

4. Inclusion of additional measurement in the study protocol

a. Because of the importance of selecting the optimal dose of isoflavones for testing the effect of soy on menopausal symptoms, it would be wise to determine serum isoflavone levels in the women consuming the soy bar to compare to the concentrations of isoflavones present in the Asian population. A small sub-set of serum samples from women on soy will be selected for this purpose. The number of samples needed to obtain statistically reliable data will be assessed by using data in two articles in the literature on levels in Asian populations and after soy intake (41, 42). This analysis will require the addition of sodium azide to the serum in order to prevent breakdown of the phytoestrogens. Additional funding would be sought for this analysis.

b. Women in our study are on the soy or placebo bars for 3 months. This time period is inadequate to expect to see differences in bone density (using DEXA) but should be adequate for determining bone status by determination of urinary measures. We are currently investigating the most appropriate determination for our study population and the costs of such an addition to the protocol. Urine samples will be collected and stored in a manner to allow assessment of bone status, if this is considered a worthwhile population to obtain this information. Additional funds will be sought to carry out this analysis. This will require the collection of two 24-hour urines.

c. In the original proposal, the serum hormones measured are estrone, estradiol, estrone sulfate, androstenedione, and follicle stimulating hormone. We would like to include the measurement of luteinizing hormone, testosterone, Free Estradiol, and serum hormone bind globulin. Additional funds will be sought for these measurements.

3. CONCLUSION:

Recruitment of our study population is now proceeding rapidly and we expect to meet our study goal by the end of the third year or early in the fourth year. Procedures have been clearly outlined in our Manual of Operations (MOOP), and all changes to the manual are dated and new pages inserted as needed. Data entry will begin shortly, and all data will be double entered and compared for accuracy. Laboratory methods have been tested and put in place. Hormone determinations will be done in batches that will include all samples from that specific subject. Sloan-Kettering has a duplicate of the MOOP and receives all updated forms or pages, when necessary. Communications between the two sites taken place at least weekly concerning recruitment, recruitment plans, development of flyers and forms, and shipment of samples. The Sloan-Kettering site was visited by Dr. Woods and Ms. Ann LaBrode on November 11, 1995 to review procedures, tour facilities, discuss recruitment, and strategize on the time table for the next 6-9 months. (See Appendix E for Agenda.) The recent use of radio in Boston has helped "catch-up" on our recruitment schedule while Sloan-Kettering is pursuing other clinic sites to increase recruitment. Recruitment from the general public will be investigated.

Additional hormone determinations are being planned to expand our possibility of observing hormone differences in the soy versus placebo group. Dr. Woods attended the 2nd International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, which was especially informative, and it enriched the discussion with the site visit team on October 9, 1996. Suggestions made by the site visit team have been incorporated into this report and into the study procedures and MOOP. The site visit was an excellent resource which we appreciated.

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5. Appendices

A. Relevant Studies of Research Group - Tufts University School of Medicine

B. Relevant Studies of Research Group - Memorial Sloan-Kettering Cancer Center

C. Protein Technology, Inc. - Dietary Bars

D. Study Personnel

E. Agenda for Visit to Sloan-Kettering, November, 1995

Appendix A: Relevant Studies of Research Group - Tufts University School of Medicine

Our research group has had a long commitment to the area of diet, hormone metabolism, and breast cancer. Earlier studies from our group concentrated on comparing dietary intake and serum hormone levels in special populations (28-30). We observed that high dietary fat and low fiber levels were associated with high serum hormone levels. In order to determine whether this was a cause and effect relationship, we began metabolic studies to control dietary intake of fat and fiber to test their independent effects on serum estrogens (31-34). Woods, et al. (33) reported a significant effect of the low fat-high fiber diet on lowering serum estrone-sulfate in seventeen pre-menopausal women.

The importance of estrogen conjugate, estrone sulfate, has been established by recent observations that it accounts for 40-50% of the estrogen present in the blood stream and has a concentration 10 times higher than that of estrone or estradiol (35). In addition, estrone sulfate has a longer half-life in serum than estrone or estradiol: 5-10 hours for estrone sulfate (35,36) compared to 1 and 2 hours, respectively, for estrone and estradiol (37). The decrease in estrone sulfate seen in our study could reflect reduced production of estrone and estradiol or a shift in the metabolism of estrone and estradiol away from sulfation toward other estrogen metabolites not measured in this study. In this regard, we recently reported that when omnivore women were shifted to this low-fat high fiber diet, there was a significant decrease in urinary excretion of the 16-hydroxylated estrogen metabolites, estriol and 16-hydroxyestrone (32). Studies with 48 premenopausal women have now demonstrated that a low fat high fiber diet results in a 30% decrease in estrone-sulfate, and approximately a 10% decrease in estrone, estradiol, and free estradiol (59). Repeated measures regression techniques indicate that fiber from a mixed food source versus fat contribute equally to the decrease in estrone-sulfate while fiber alone accounts for the major decrease in estradiol. Therefore, both fat and fiber have been shown to effect not only the amount but also the metabolism of endogenous estrogens (Table 1) (34).

Table 1

The Independent Effects of Dietary Fat and Fiber on Serum Hormone Concentrations

	FAT		FIBER	
Hormone	% change	p value	% change	p value
Estrone	-6.8	0.12	-4.7	0.27
Estrone Sulfate	-18.1	<0.001**	-17.1	0.002**
Estradiol	-2.2	0.71	-11.0	0.045**
Free Estradiol	-8.4	0.17	-4.8	0.43
Testosterone	-8.1	0.13	-0.7	0.90
Androstenedione	-8.5	0.018**	-3.6	0.35
SHBG	-3.6	0.39	-11.2	0.005**

* A negative sign indicates that the concentration of hormone decreased after consuming a diet in which either fat was reduced from 40% to 20% or 25% of total calories, or fiber was increased from 12 grams to 40 grams/day.

** Statistically significant

n=48 subjects; 310-332 individual measurements per hormone (data include all protocols)

We have recently published (38) the results of our study of dietary intervention in African American women. This study used the same protocol as the above study of premenopausal Caucasian women. The same dietary changes to low fat and high fiber resulted in decreases in E_1, E_2, and E_1S0_4 in this population as observed in the Caucasian women. However, an increase in Androstenedione was observed going from the high fat-low fiber to the low fat-high fiber diet that we did not observe before. In addition, the African American women had higher baseline hormone values than the Caucasian women, by as much as 50% even when corrected for age, parity, and BMI.

Co-Investigator, Dr. Barnett is carrying out a cross sectional study on pre and post menopausal women of Caucasian and African-American background to evaluate the effect of body fat distribution on endogenous hormone values.

Research studies currently under investigation include work on the effect of different types of fiber on serum estrogens to verify and expand the work reported by Rose, et al (39) in which he reported a positive effect of wheat bran in decreasing serum estrogens in contrast to a lack of effect by oat or corn bran. Our investigation is using fiber sources from wheat, legumes, vegetables, and fruits in quantities of 25 grams 7 fiber per day to determine their impact on endogenous hormones using female college students in a cafeteria setting (40). The dining service has cooperated to prepare and serve specially designed diets to the study participants. We did not observe a decrease in endogenous hormones with wheat fiber but legumes (low in phytoestrogens) did result in a decrease in serum gonadotropins (unpublished data).

Currently, Dr. Goldin is studying the effect of feeding pre and postmenopausal women food sources rich in isoflavanoids and lignans (phytoestrogens) and measuring the effects of these individual food products on the concentration of these compounds in urine and plasma. Urinary metabolites of phytoestrogens from these participants are being isolated and used to test their effect on the growth of breast cancer cells in culture and the ability of these metabolites to act either antagonistically or synergistically with estrogens is also being investigated. Preliminary results from these studies indicate that food sources rich in phytoestrogens significantly increase the concentration of these compounds in the urine. In addition, it appears that ingestion of phytoesterogens lowers the urinary excretion of a number of estrogens.

Our laboratory has received funding to investigate the effect of a dietary soy bar or placebo bar to change menopausal symptoms in two different populations that we had not studied previously: 1.) Women who have had breast cancer, 2.) Women without personal or family history of breast cancer. This study is only three months long, and women are randomized into either a soy or placebo group. They are blinded as to their assignment. Menopausal symptoms and hormone levels are collected as described for the current study. Sixty women are being recruited for each group. This data will allow us to compare the response of the these groups to the soy and placebo intervention and collect additional baseline data on reporting of menopausal symptoms. This population will also contribute additional data on the correlation of hormone levels and reporting of menopausal symptoms.

Appendix B: Relevant Studies of Research Group - Memorial Sloan-Kettering Cancer Center Dr. Ruby Senie

The Metropolitan New York Registry of Breast Cancer Families

The Metropolitan New York Registry of Breast Cancer Families will be a subset of the Cooperative Family Registry for Epidemiologic studies of Brest Cancer (CFRBC). The proposed multi-institutional project will enable recruitment of a culturally diverse population of breast and ovarian cancer patients, unaffected individuals with a strong family history, and their relatives to the Metropolitan New York Registry. This collaborative project joins the expertise and enthusiasm of scientists from seven major New York medical centers with members of voluntary and community-based organizations. Families will be recruited through the clinical setting as well as through outreach programs of voluntary organizations and community groups located in the five boroughs of New York City, Nassau, and Suffolk Counties. An estimated 1,681 families will be eligible for recruitment to the Registry. We anticipate enrolling 1,022 families with an average of composition of 6 members including the proband who provided the initial family history. All male breast cancer cases diagnosed in any collaborating medical center will be invited to enroll int he Registry and all female blood relatives of participating probands age 18 or older will be recruited. All consenting participants will be asked to provide blood and urine specimens for banking; personal and dietary data will be collected through telephone interview. Participants will be followed annually by mail. In addition, biannual newsletters will include current research findings and recommendations. Educational programs will be conducted in community settings; a video tape introducing breast cancer genetics will be provided. Than you cards will be mailed soon after enrollment and birthday cards from the Registry will express the collaborative environment established between Registry participants and scientific collaborators.

Residents of Long Island Health Study

Protocol Summary:

This study is a collaborative effort among New York City and Long Island researchers to determine whether environmental contaminants (primarily organochlorine pesticide compounds (OCC) and polycyclic aromatic hydrocarbons (PAH), ubiquitous carcinogens) increase the risk of breast cancer among women on Long Island, New York. Concern about this issue among Long Island residents led to federal legislation mandating such a study.

This investigation will be a four-year, collaborative, population-based, case-control study to identify environmental risk factors for breast cancer among women on Long Island. Cases will be residents of Nassau and Suffolk counties in New York State who are over 20 years of age, and are diagnosed with primary in-situ or invasive breast cancer during a 12-month period. Cases will be identified through daily review of pathology/cytology or tumor registry records located at some 40 hospitals throughout Long Island and New York City, and other rapid reporting methods. Population-based controls will be frequency matched to cases by 5-year age groups, and identified using random digit dialing (RDD) and Health Care Finance and Administration (HCFA) rosters.

Objectives:

The primary aim of this funded case-control study is to determine whether certain environmental exposures (primarily organochlorine pesticide compounds (OCC) and polycyclic aromatic hydrocarbons (PAH) are associated with the risk of breast cancer among a population-based sample of Long Island women. Exposure assessment includes biologic specimens (blood and urine), home sampling (soil, water, and dust), in-person interviews (including residential and occupational histories), and geographical modeling of historic exposure. Other established and suspected risk factors for the disease will also be evaluated.

This proposal is a collaborative effort among New York City and Long Island researchers to determine whether environmental contaminants increase the risk of breast cancer among women on Long Island, New York. Recently, several studies suggest that certain environmental exposures, primarily some organochlorines (commonly used as pesticides), affect estrogen production under laboratory conditions, increase mammary tumors in animals, and may increase a woman's risk of breast cancer. Possible routes through which organochlorine compounds (OCC) may affect breast cancer risk include estrogen metabolism. Further studies have linked residence near hazardous waste sites or chemical facilities with breast cancer risk. Although the exact exposures have not been identified, putative agents derived from such exposures may include polycyclic aromatic hydrocarbons (PAH), ubiquitous mammary carcinogens that are estrogenic in some *in vitro* test systems. Electromagnetic fields (EMF) on the job have been shown to increase breast cancer risk among men, and residential EMF exposure has been hypothesized to increase risk among women. The primary aim of the proposed study is to determine whether OCC (DDE, PCBs, chlordane), PAH and EMF are associated with breast cancer risk among Long Island women.

Appendix C: Protein Technology, Inc. - Dietary Bars

Supro Food Bar, Protein Technology, Inc. (St. Louis)
Chocolate Flavor - Uncoated

The Supro Food Bar was developed for use in human clinical studies. The bar has been designed to provide 15 grams of protein from Supro per serving.

Supro Food Bar - Chocolate Flavor provides many health benefits:

* Excellent Source of Protein
* Contains Isoflavones
* Same Vitamins & Minerals as Milk
* Excellent Source of Calcium

TUFTS UNIVERSITY - BARS - First Production

		per 100g:		per serving:		% DV	
	Units	Uncoated 675HG 2656-18-2	Uncoated Placebo 2656-18-3	Uncoated 675HG 2656-18-2	Uncoated Placebo 2656-18-3	Uncoated 675HG 2656-18-2	Uncoated Placebo 2656-18-3
Serving	g			56	56	56	56
Moisture	g	11.6	11.9	6.5	6.7		
Fat	g	2.28	1.66	1.3	0.9	2	2
Protein	g	27	29.2	15.1	16.4	30	30
Ash	g	3.55	3.77	2.0	2.1		
CHO (by diff)	g	55.57	53.47	31.1	29.9	10	10
Calories	Kcal			196	194		
Ca	mg	1270	887	711	497	70	50
Fe	mg	7.1	3.67	4.0	2.1	22	11
P	mg	897	542	502	304	50	30
Na	mg	300	190	168	106	7	4
Folic Acid	mcg	254	283	142	158	40	40
Pan Acid	mg	1.68	1.39	0.94	0.78	10	10
B6	mg	0.47	0.406	0.26	0.23	10	10
B2	mg	0.797	0.628	0.45	0.35	25	20
B1	mg	0.415	0.258	0.23	0.14	15	10
Vit A	IU	1193	1444	668	809	15	15
B12	mcg	2.3	5.7	1.29	3.19	20	50
Vit C	mg	2.51	2.98	1.41	1.67	2	3
Vit D	IU	274	292	153	164	40	40

Soy Bar Ingredients:	Supro brand isolated soy protein, corn syrup, high fructose corn syrup, rice syrup solids, cocoa (processed with alkali), glycerine, vitamins and minerals [tricalcium phosphate, dimagnesium, phosphate, ascorbic acid (vitamin C), vitamin A palmitate, zinc oxide, vitamin D3, cyanocobalamin (vitamin B12), calcium pantothenate, riboflavin (vitamin B2), niacinamide, pyridoxine hydrochloride (vitamin B6), thiamine mononitrate (vitamin B1), and folic acid], artificial vanilla flavor, and salt.
Placebo Bar Ingredients:	Corn syrup, whey protein concentrate, high fructose corn syrup, rice syrup solids, calcium sodium caseinate, cocoa (processed with alkali), glycerine, vitamins/minerals [calcium phosphate, dimagnesium phosphate, vitamin C (ascorbic acid), vitam A palmitate, zinc oxide, vitamin D3, vitamin B12 (cyanocobalamin), calcium pantothenate, vitamin B2 (riboflavin), niacinamide, vitamin B6 (pyridoxine hydrochloride), vitamin B1 (thiamine mononitrate), and folic acid], natural and artificial flavors, and salt.
Phytoestrogen Content of the Protein Technology International Soy Bar:	The phytoestrogen content of the soy bar is based on the isoflavone profile of the soy isolate being used as the main ingredient. A 56 gram bar, with 15.1 gm of protein from supro soy protein isolate, contains 15 mg of genistein and 9 mg of diadzien or 24 mg of phytoestrogens per bar. The supro soy protein isolate is analyzed for phytoestrogen content by Protein Technologies International as part of their quality control in producing Supro (a time mark product). The isoflavones are present as the conjugates and the processing used to form the bars is not expected to change the isoflavone content. Protein Technologies International will carry out separate analysis of isoflavone content of our bars for each batch sent. In addition, we are keeping 3 bars/shipment of soy and placebo for this purpose if deemed necessary at a later date. These samples can be analyzed for effects of time on the phytoestrogen content. The one year shelf life given by Protein Technologies International is related to consumer acceptability essentially regarding the texture of the bar. With aging, some hardening of the bar takes place.

Study Personnel

Boston: Tufts University School of Medicine

Dr. Margo Woods, Principal Investigator
Dr. Sherwood Gorbach, Co-Investigator (No Salary)
Dr. Barry Goldin, Co-Investigator
Dr. Donna Spiegelman, Biostatistician
Ms. Ann LaBrode, Project Director

New York City: Sloan Kettering

Dr. Ruby Senie, Principal Investigator, Sub-contract
Ms. Suzanne Tenser, Project Director
Dr. Freddie Kronnenberg, Co-Investigator (Consultant)

APPENDIX E: AGENDA FOR VIST TO SLOAN-KETTERING, 11/95

AGENDA
Menopausal Symptoms Study
Friday November 11, 1995

10:00 Tour of Facilities
- Clinic/Recruitment Area/Participant Interview Area
- Laboratory/Specimen Processing Area/Specimen Storage

10:30-11:15 Recruitment
- Current Number of Participants/Control & Intervention
- Recruitment Goals - 3 women/month
- Recruitment Strategies/Implementation (see separate sheet)
- Stipend

11:15-12:00 Paradox
- Demonstration Set up on Computer
- Demonstration of entering forms
- Transfer of Data

12:00-1:30 Review Study Protocol-Manual of Operations (Margo, Ann, Ruby, Suzanne)
- Screening/Enrollment/Questionnaires
- Distribution and labelling of Supplement bars
- Tracking of Participants - ACT by Symantec
- Labels for forms/bloods/urines
- Current Literature/Developing a bibliography

1:30-2:15 Processing of Laboratory Samples (Demonstration Ann, Suzanne))
- Blood
- Urine
- Logging in Samples
- Labeling of Samples
- Storage and Shipment of samples

2:15-3:00 Keeping a Food Record (Ann, Suzanne)
- Instruction
- Practice
- Documenting a Food Record
- Dietary Assessment Training Manual/Certification

Budget (Margo, Ruby)

3:00-4:00 Other Issues

4:00 ADJOURN